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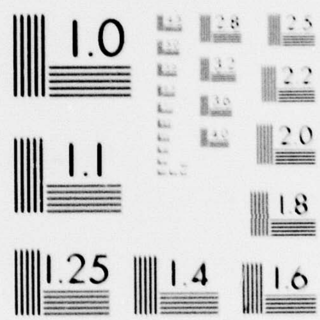

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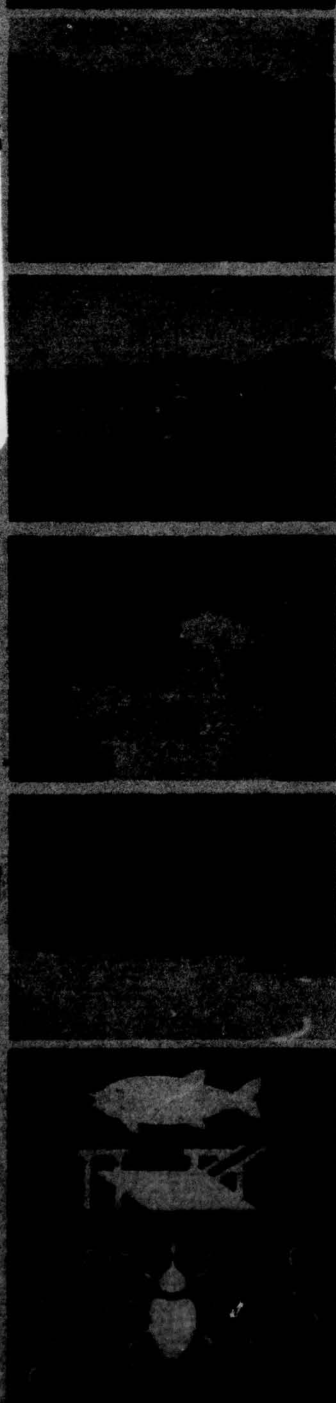

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INSECTS FOR BIOLOGICAL CONTROL OF AQUATIC PLANTS

By T.D. Center

U.S. Department of Agriculture
Aquatic Plant Management Laboratory
Fort Lauderdale, Fla. 33314

August 1979

Final Report

Prepared for Office, Chief of Engineers, U. S. Army
Washington, D. C. 20314

Under USDA/SHEA Agreement No. 12-14-700-995

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Sameodes albiguttalis (Warren) is a pyralid moth currently under study for the biological control of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms-Laubach). The objectives of these studies are to: (a) establish field populations of this insect, (b) estimate its vagility, and (c) determine its efficacy in controlling the target weed. Progress made in realizing these objectives includes: (a) the establishment of a greenhouse colony as a stock

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for field releases and (b) the actual release of several hundred insects. To date, 1477 infested waterhyacinth plants have been transplanted to three sites as well as 37 adult insects and 154 first instar larvae. It is still too soon to ascertain the success or failure of these releases and successive releases are continuing.

Preface

This report presents results of a biological control program being conducted for the Aquatic Plant Control Research Program (APCRP) by the United States Department of Agriculture (USDA), Science and Education Administration, Aquatic Plant Management Laboratory, Fort Lauderdale, Fla. The purpose of this program is to evaluate insects to determine their potential for use in aquatic plant control. This particular project in the overall program involved the field release of *Sameodes* against waterhyacinth and the domestic survey of insects on hydrilla and Eurasian watermilfoil. Funds for this effort are provided by the Office, Chief of Engineers, under appropriation number 96X3122, Construction General, through the APCRP at the U. S. Army Engineer Waterways Experiment Station (WES).

The principal investigator for the work was Dr. T. D. Center, USDA, who prepared this report.

The work was monitored at WES by Messrs. W. N. Rushing and R. F. Theriot of the Aquatic Plant Research Branch (APRB), under the general supervision of Mr. W. G. Shockley, Chief of Mobility and Environmental Systems Laboratory (MESL), and Mr. B. O. Benn, Chief of the Environmental Systems Division, and under the direct supervision of Mr. J. L. Decell, Chief of the APRB. As a result of a reorganization at WES, Mr. Decell is now manager of the APCRP, which is a part of the Environmental Laboratory of which Dr. John Harrison is Chief.

The Commander and Director of WES during this period was COL John L. Cannon, CE. Technical Director was Mr. F. R. Brown.

Contents

INSECTS FOR BIOLOGICAL CONTROL OF AQUATIC PLANTS

Project A: Release and Establishment of *Sameodes albiguttalis*; Monitor Dispersal; Evaluate Efficacy

1. *Sameodes albiguttalis* (Warren) was first studied in Uruguay in 1965 for its potential as a biological control agent of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms-Laubach). It was more intensively studied at the U. S. Department of Agriculture (USDA) Laboratory in Hurlingham, Argentina, from 1973 to 1977 to determine its host specificity, ecology, life history, and efficacy in controlling waterhyacinth. It was subsequently considered safe for introduction into the United States and received into quarantine at Gainesville, Florida. Approval for the release of this insect was requested from and granted by the Interagency Working Group on the Biological Control of Weeds (IAWGBCW) in 1978. Following concurrence by various State agencies in the southeast, *Sameodes albiguttalis* was released at three locations in Florida in October 1977.

2. The original three Florida release sites (Edgar, St. Petersburg, and Fort Lauderdale) were monitored repeatedly for several months for evidence that the insects had become established. No such evidence was obtained and the releases were considered unsuccessful. This was discussed at a meeting between the U. S. Army Engineer Waterways Experiment Station (WES) and USDA, and three primary needs were identified to enable the implementation of this insect in a management program. The needs designated were as follows:

   a. To establish field populations of *Sameodes albiguttalis* and to develop a methodology for its release and establishment.

   b. To determine the vagility (ability to disseminate) of this insect so that appropriate release strategies could be developed.

   c. To evaluate the efficacy of this insect as a control of waterhyacinth.

3. As these needs were identified, they were translated into research objectives in a work statement to be met by the end of FY 79. The work statement was partitioned into four phases with a total of 17 tasks. The following is a report of the progress made in carrying out these tasks in FY 78.

Phase I: Establish a laboratory colony of *Sameodes albiguttalis*

4. This phase consisted of three primary tasks that were to have been completed by July 1978. The first task was the preparation of a greenhouse for rearing a colony of insects. This involved the repair of an evaporative cooling system to prevent detrimentally high temperatures and the construction of specially designed cages and tanks for culturing both insects and waterhyacinth.

5. In initiating a colony of the insects researchers were faced with the problem of confining them to a small area while the population was low but, at the same time, allowing sufficient space and plant material for them to expand into as the population increased. As a solution to this problem, cages were constructed that were fitted to three standard greenhouse benches. The bases were made of 2.54 by 30.48 cm redwood in the form of 0.91- by 1.22- by 3.30-m open-topped boxes. These were coated with polyurethane, lined with polyethylene plastic film, and filled with water. Each was then filled with waterhyacinth and fertilized with Peter's 20-20-20. Frames were constructed of 1.9-cm polyvinyl chloride (PVC) pipe which was inserted into each base and extended 1 m above the bottom. The frames were covered with nylon screening. Zippers were affixed to the screen at each side so that the side panels could be removed. Adjoining cages could then be attached to one another to expand the area enclosed by the cage up to that of the greenhouse bench. This permitted sufficient flexibility to confine the insects to a 1.11-m^2 area or to allow them to expand into an area up to 8.92 m^2. Eighteen cages were built on the three greenhouse benches giving a rearing area of 20 m^2 (two benches with 5.57 m^2 each and one bench with 8.92 m^2).

6. The second task was to obtain a stock of insects from the colony at the Gainesville USDA quarantine facility. Two shipments of

29 and 53 pupae, which were hand carried from Gainesville, served as the basis of the colony. Of this total of 82 pupae, 27 were received dead. Forty-two adults were reared from the fifty-five living pupae for a total pupal emergence of 76 percent. The adult moths were paired and placed in petri dishes with moist filter paper on the bottom and a waterhyacinth leaf. A portion of the epidermis was stripped from the leaf to provide suitable oviposition sites for the females. Observations revealed that the females walk over the leaf waving the tip of their abdomen over the surface until they find a lesion or abraded area. They then probe this area with their ovipositor and, if the lesion is suitable, they force the eggs into the spongy aerenchyma. Stripping a small area of the leaf epidermis seemed to enhance oviposition by increasing the potential number of suitable sites. The leaves in each dish were checked daily for oviposition. From these a total of 2464 eggs (an average of 103 eggs/female) were obtained. This provided a sufficient quantity of insects for the basis of a colony, thereby completing the second task.

7. The third objective was the establishment of these insects in the greenhouse and the production of sufficient numbers to attempt the initial field releases. The eggs obtained from the Gainesville stock were held in petri dishes until the larvae eclosed. From the initial 2464 eggs, a total of 2093 larvae were obtained, indicating a total emergence of 85 percent. The first instar larvae were transferred to individual cages in the greenhouse (ca. 262 larvae/cage). As they developed and the population increased, the condition of the plants was monitored closely. As the plants deteriorated, fresh material was added and adjacent cages were opened up to allow the colony to expand. This continued until all 18 cages in the greenhouse were infested with larvae. By repeatedly removing a portion of the colony and re-adding fresh plants, there was no difficulty in perpetuating a large colony of insects. The primary problem was the determination of the proper point at which to harvest and restock before the condition of the plants deteriorated to such an extent that the vigor of the colony was adversely affected. This point seemed to be at the time a large amount of damage

was apparent but before the plants began to wilt.

Phase II: Release and establishment of *Sameodes* at field sites

8. The primary goal of this project was the establishment of large field populations of *Sameodes* and, by so doing, to shift an implementation program for waterhyacinth control from the laboratory to the field. Rather than to make many small, widely scattered releases, it was deemed more prudent to concentrate the insects in a few sites until self-perpetuating populations were obtained. The ultimate goal was to establish local breeding populations within a confined geographical zone across the southern part of the State of Florida. From these populations, dispersal in a northward direction could be determined by monitoring at specific check points. Once this information was obtained the available manpower could be utilized in the most expeditious manner to make further strategic releases that would take maximum advantage of the vagility of the insect.

9. As a prelude to the above goals, it was necessary to select appropriate sites. The criteria used in selecting these sites were: (a) they must be fairly isolated and protected, (b) they must be fairly equally spaced from one another and generally in an east-west line, and (c) they must have a continuous and permanent waterhyacinth population. Three such sites were selected at approximately 26°N latitude. The sites were as follows:

Site 4-S. Copeland, Collier County, Florida. Drainage stream from Fakahatchee Strand.

Site 5-S. Orchid Isles, Collier County, Florida. Backwater section of Gator Hook Strand along Tamiami Trail.

Site 6-S. Miami Canal, Broward County, Florida. Conservation Area 3, along Levee 67A.

10. As the greenhouse colony increased, portions of the infested plants were removed. These portions were divided into three lots, and one lot was transplanted into each of the three sites. A portion of each lot was initially placed in a 1- by 1- by 1-m nylon screen cage and a portion was left uncaged. Other methods of releases attempted included caged adults and direct release of first instar larvae. The dates and types of releases are as follows:

Date	Site	Type of Release
01 June 1978	4S	35 plants in cage
	5S	34 plants in cage; 30 plants outside cage
02 June 1978	6S	28 plants in cage; 36 plants outside cage
09 June 1978	5S	6 adult females, 11 males in cage
13 June 1978	6S	7 adult females, 13 males in cage
23 June 1978	4S	96 plants outside cage
	5S	78 plants outside cage
	6S	90 plants outside cage
30 June 1978	4S	150 plants outside cage
	5S	150 plants oustide cage
	6S	150 plants outside cage
28 July 1978	4S	300 plants outside cage
	5S	300 plants outside cage
05 Sept 1978	6S	154 first instar larvae placed on small plants

Site Totals	
4S	581 infested plants
5S	592 infested plants; 6 females, 11 males
6S	304 infested plants; 7 females, 13 males; 154 first instar larvae

11. The use of cages in the sites has largely been abandoned because they tend to draw attention to the release area, which results in varying degrees of disturbance. These will continue to be used, however, in releasing the adult insects. The cages did not seem to improve the larval releases. Each site was closely monitored throughout the summer for signs that the insects had become established. A summary of observations for each site follows:

Site 4S
- 09 June - Cage knocked over by a large rock. No *Sameodes* found.
- 23 June - No *Sameodes*.
- 30 June - No *Sameodes*.

Site 4S (Continued)	28 July -	One dead pupa and eight empty cocoons found in plants from prior release.
Site 5S	09 June -	No *Sameodes*.
	23 June -	No *Sameodes*. One plant with apparent feeding.
	30 June -	Larvae found in plants placed out previous week. One adult female found in middle of site well away from release point.
	28 July -	No *Sameodes*.
Site 6S	13 June -	Fresh damage apparent in plants from previous release.
	23 June -	First instar larvae and pupae found.
	30 June -	Ten pupae and three fourth instar larvae found.
	28 July -	One larvae and one pupa found. Plants appeared to have been recently sprayed. All plants dead by early August.
	18 Sept -	Several large larvae found in immediate area of 05 Sept release.

12. In summary, evidence that the insects had persisted in the release area for a short period was obtained at all sites. Generally, this evidence consisted of immature stages in the plants placed out in the previous release. In spite of the fact that one adult female was collected well away from the release point, no evidence was found of larval infestation in plants other than those introduced. It is suspected that the adults are moving to a different area, perhaps to a different growth form of the plant, to oviposit. This possibility is being investigated now. Based on past experience, however, it is far too early to ascertain the success or failure of the releases. It is extremely difficult to find a small population of insects in the tremendous mass of waterhyacinths present at each site. It is encouraging to note that the first instar larvae placed on small "wild" plants at Site 6S have survived at least 2 weeks. It is still too early, however, to call this a successful establishment.

13. At this point, Phase II is not yet completed. The successful completion of this phase is contingent upon the definite establishment

of the insects in the primary sites. This phase will be continued until the insects are firmly established.

Phase III: Monitor long-range dispersal of *Sameodes*

14. This phase of the project is dependent upon the existence of dispersing populations of the insects. This phase has not yet begun due to a lack of these populations.

Phase IV: Evaluate efficacy of *Sameodes* in suppressing waterhyacinth growth

15. As in the previous phase, this phase is dependent upon an existent population of *Sameodes*. Since the populations are not yet established, this phase has essentially not yet begun. However, a small population has been discovered in St. Petersburg from the release made in October 1977. Two larvae and one pupa were found approximately 10 m from the release point on 15 June. This site was checked again on 23 August and no insects were found in the release area. Upon checking a fringe of plants at the end of a drainage canal several hundred meters from the release point, however, a significant population was found. The plants the insects were found on were generally the small bulbous type, although a few were found on the larger plants. A transect has now been established in this canal and evaluation of the effect of this population has begun. The first samples were taken 7 September and have not yet been completely analyzed.

Discussion

16. The research on the release, establishment, and dispersal of *Sameodes albiguttalis* is going as planned if somewhat behind schedule. Based upon the fact that 8 months were required before the insects were found in St. Petersburg, it may be quite a while before the success of the current releases is apparent. The time schedule was established on the premise that the first insects released would become established. This was more of an optimistic hope than a realistic goal. It is now apparent that *Sameodes* will not easily become established and that research efforts will have to be concentrated towards this end. This will mean that the initiation of Phase III may have to be advanced several months. Phase IV will be continued at the St. Petersburg site (2S).

17. The fact that the insects at St. Petersburg were found some distance from the release point lends credence to the hypothesis that they are moving to more suitable plants. Previous data from South America suggest that they may prefer the small, bulbous, petioled growth form of waterhyacinth. The observations at St. Petersburg and at site 6S also lend credence to this hypothesis. During the next few weeks experiments will be conducted with releases on different forms of the plant to determine if there is, indeed, any preference.

18. A major limiting factor in the release program is the availability of significant quantities of insects. Once the colony is thinned, it may take several weeks before it rebuilds to a point where another release is possible. In addition, it is difficult to obtain sufficient numbers at any one time for anything other than fairly small releases. To remedy this, the research team is presently stocking a small pond on the Agricultural Research Center grounds with waterhyacinth and the insects. While this constitutes a fourth release, it is not greatly inconsistent with the line of establishment. It is hoped that by having a site on station which can be readily manipulated, a large source of insects can be obtained for other releases.

Project B: Domestic Survey of Insects on *Hydrilla verticillata* and *Myriophyllum spicatum*

19. Of the aquatic weeds, the submersed forms are, by far, usually the most difficult and costly to control. Two introduced species, Eurasian watermilfoil (*Myriophyllum spicatum*) and hydrilla (*Hydrilla verticillata*), account for the majority of the problems with submersed weeds. These weeds are currently controlled by chemical herbicides or mechanical harvesting. Chemical treatment, while now commonly used, is usually restricted to areas where the water will not be used for drinking purposes. Both methods are expensive, are only temporarily effective with retreatment almost always being necessary, and are effective only in the area of treatment. The use of natural enemies, which has proven to be effective for controlling a variety of terrestrial weeds

(and also the aquatic alligatorweed *Alternanthera philoxeroides*), overcomes these problems.

20. Typically, a program of biological control involves searching for organisms that stress the target pest in the native range of the pest, evaluating the potential of the organism for stressing the pest in its adventive range, testing promising biocontrol agents for host specificity, and, finally, importing and establishing populations of the biocontrol agent within the adventive range of the pest. While re-associating a target pest with its natural enemies from its area of endemism is the classical biological control approach, there are additional sources for potential biological control organisms. For example, exotic species may already be established in this country, perhaps by having been introduced simultaneously with the target pest. This introduced organism could serve as a biological control of the pest and would avoid the costs and delays involved in discovering and testing a suitable foreign organism.

21. Indigenous organisms could also be utilized as biological control agents. This, however, may require the manipulation of biotic and environmental factors that are currently keeping the indigenous organisms from controlling the submersed pests. However, an indigenous organism may, theoretically, be superior to an introduced biocontrol agent since the host (the target submersed weed) has not had time to evolve defensive mechanisms against this indigenous species. Thus, when the introduced submersed pest becomes established within the range of this indigenous herbivore species, effective control of the pest could result.

22. The waste of money and effort in finding and testing an organism for controlling a weed in a particular area when the organism is already established in that area is obvious. A thorough, domestic survey of the fauna associated with *M. spicatum* and *H. verticillata* would help ensure against this occurrence. Coupled with laboratory tests and search of pertinent literature, this domestic survey should also be able to detect indigenous species that might serve as biocontrol agents on these two weeds.

23. In recognition of this, personnel at a joint meeting of WES and USDA Aquatic Weed Control developed a work statement that included a domestic survey project whose objective was to compile a list of organisms associated with submersed aquatic weeds in southeastern United States by the end of fiscal year 1980.

Technical problems

24. Although the entomologist in charge of this survey was hired a month later than anticipated in the work statement, progress on the project is relatively on schedule. The primary obstacle during the start-up phase of this project has been the lack of collecting equipment and vials, microscopes, slide-making equipment, identification guides, and other pertinent literature. The necessary equipment has been ordered and is now beginning to arrive.

25. The difficulty of finding and removing the organisms on these submersed weeds is currently creating a bottleneck. One technician works full time examining field-collected hydrilla and Eurasian watermilfoil under a dissecting microscope and removing the organisms on or in these weeds. The microscopic examination of relatively small samples is extremely time-consuming and highly labor intensive.

26. Transporting and storing weed samples in such a manner that the organisms on them are maintained in good condition has proven occasionally to be a problem. The environmental chambers used to store samples often do not maintain a steady temperature and occasionally the samples become frozen. This eliminates the possibility of successfully rearing or testing any of the organisms in the sample.

Results

27. As outlined in the work statement, this project consists of nine tasks divided into two phases, a qualitative and a quantitative phase. The qualitative phase is approximately on schedule. Sites infested with one or both of the weeds have been found by contacting other aquatic researchers. A weed sample is collected by use of a potato rake with an attached rope, with an aquatic insect net, or, occasionally, by hand. The weed is placed in a numbered plastic bag which is then placed in a cooler for transportation back to the laboratory. Physical

and chemical characteristics of the collecting site are noted. The weed is examined for insects in the laboratory; the insects are removed and preserved. Attempts are made to rear some immature forms. A relative rating for the degree of damage to the weed is also assigned at this time. The dry weight of the weed searched is then determined. The insect specimens from each collection are sorted into different taxa and then stored pending final identification to species level. As of mid-September, collections from 24 sites in 12 counties have been obtained.

28. The quantitative phase of this project is also approximately on schedule. A list of sites has been compiled. The sites which will be regularly sampled will be selected from this list once a quantitative sampler is constructed.

29. A rather thorough search of the literature on aquatic samplers has not revealed any which would be suitable for this study. The sampler needed must reliably sample a given portion of submersed weed, retaining both the weed and the organism on or near the weed inside the sampler. This sampler should work well at variable depths, at least within the range of 0.5 to 2+ m. Additionally, the sampler should be portable and capable of being used by one person without specially equipped boats, etc. Since none of the samplers used by other researchers or described in the literature meet these criteria, a new sampler was designed and is currently being constructed.

Discussion

30. The process of selecting sampling sites has, coincidentally, indicated the lack of knowledge of the limits of the distribution of these two weeds, both inside Florida and elsewhere. Most researchers are aware of infestations in major lakes, rivers, and canals. However, these sites frequently receive chemical treatments, which usually degrade their suitability for study. Even from just the few early samples, a very definite lack of fauna has been noted from sites that had received recent chemical treatment.

31. The distribution of Eurasian watermilfoil in Florida is rather limited. Samples have been taken from Lake Seminole and Crystal River, the only two Florida locations for *M. spicatum* recorded in the literature.

32. Preliminary examination of the insects found on hydrilla allows some generalizations. The aquatic entomofauna associated with hydrilla is much less abundant and probably less diverse than that associated with floating macrophytes such as waterhyacinth. Hydrilla plants from areas recently treated with herbicides are almost completely devoid of insects. While midge larvae (chironomidae) are abundant, they do not comprise 75 to 80 percent of the insects collected (the figure cited in the few literature records of the fauna on hydrilla), except in a few cases. While there is a possibility that a chironomid may be a good candidate for the biological control of hydrilla, early observations indicated that the species being collected use hydrilla simply as a substrate rather than a food source.

33. The major herbivore damage observed so far has been caused either by snails, which are frequently abundant in number and species, and by the larvae of the aquatic pyralid moth, *Parypoynx diminutalis*. This species, not thought to be in the United States until about 5 years ago, was previously only collected in the Fort Lauderdale area. It has now been collected from several south Florida counties and some sites in the Tampa area. The larva of *P. diminutalis* utilize the leaves of hydrilla for food and for constructing hibernacula. In hydrilla grown in outdoor concrete aquaria at Fort Lauderdale, the population levels of *P. diminutalis* frequently become quite high. At these high levels the plant is completely defoliated and only clumps of bare stems remain. This degree of damage has not yet been observed in the field. A pilot experiment has been set up to try to determine if it is perhaps some species of fish that reduces the population levels of *P. diminutalis* in the field.

Plans for FY 1979

34. Project A. During FY 79 researchers will continue to make releases of *Sameodes albiguttalis* at primary as well as secondary sites in an attempt to establish several populations in the region of south Florida bounded by SR 838 (Alligator Alley) on the north and US 41 (Tamiami Trail) on the south. As these populations become established, researchers will shift emphasis toward monitoring dispersal and efficacy.

In general, the time schedule originally proposed will hold true although some phases will be protracted.

35. Project B. This project is proceeding on schedule and should continue to do so through FY 1979.

36. Project C. This project constitutes foreign surveys for natural enemies of *H. verticillata* and *M. spicatum* (new project). A primary need in developing a biological control program for these two aquatic weeds is an extensive preliminary survey of the fauna associated with them throughout their native range. The range of these species is tremendous and a thorough survey will require extensive world-wide travel. It is important that this be initiated as soon as possible to delineate the most promising geographical areas for more detailed research.

37. In order to meet this need a preliminary survey of *H. verticillata* and *M. spicatum* has been proposed for 1979. The regions surveyed will include Europe, Asia Minor, India, Southeast Asia, and Australia. In addition, a contract will be negotiated to conduct similar research in Africa. This survey will cover the major accessible areas of the range of the two species and thereby provide information for directing a future program.

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